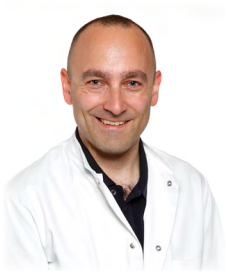
Exploring the proteome of diabetic macular edema


Lasse Jørgensen Cehofski[1,2]


Henrik Vorum[3,4]


Jakob Grauslund[1,2]

1. Department of Ophthalmology, Odense University Hospital, Odense, Denmark
2. Department of Clinical Research, University of Southern Denmark, Odense, Denmark
3. Department of Ophthalmology, Aalborg University Hospital, Aalborg, Denmark
4. Department of Clinical Medicine, Aalborg University, Aalborg, Denmark

Diabetic macular edema (DME) is a sight-threatening complication with a complex multifactorial pathophysiology. Clinicians continue to face challenges in managing DME, including the recurrence of edema, insufficient improvement of visual acuity, and limited absorption of edema despite relevant treatment. Patients with diabetes require continual follow-up with their health care providers, and the development of clinically significant DME is associated with even more visits. Understanding the basic science of DME is a cornerstone for successful management and the development of personalized treatment. At EASDec 2021, we presented our novel approach to better understand DME through advanced proteome analyses.

The proteome refers to the entire set of proteins in a cell, tissue, or body fluid. A proteomic analysis aims at quantifying all proteins in a sample, specially focusing on which proteins change with the disease being studied. The proteome analysis is not limited to a small number of "usual suspects" that are already known to be involved in the formation of DME. On the contrary, proteome analysis can provide the big picture of molecular mechanisms contributing to the development of DME through the identification of more than 1,000 different proteins in ocular fluids.

We have previously shown that the aqueous proteome humor widely reflects pathological changes in retinal vascular disease.[1] A unique donation from our collaborators at the Department of Ophthalmology, Kyoto Prefectural University of Medicine, recently allowed us to perform proteomic analysis for DME. The samples consisted of aqueous humor from treatment-naïve patients with DME and an age-matched control group.

Proteomic analysis of the samples is a multi-step workflow centered around the mass spectrometer (**Figure 1**). Successful proteomic experiments require careful sample preparation prior to mass

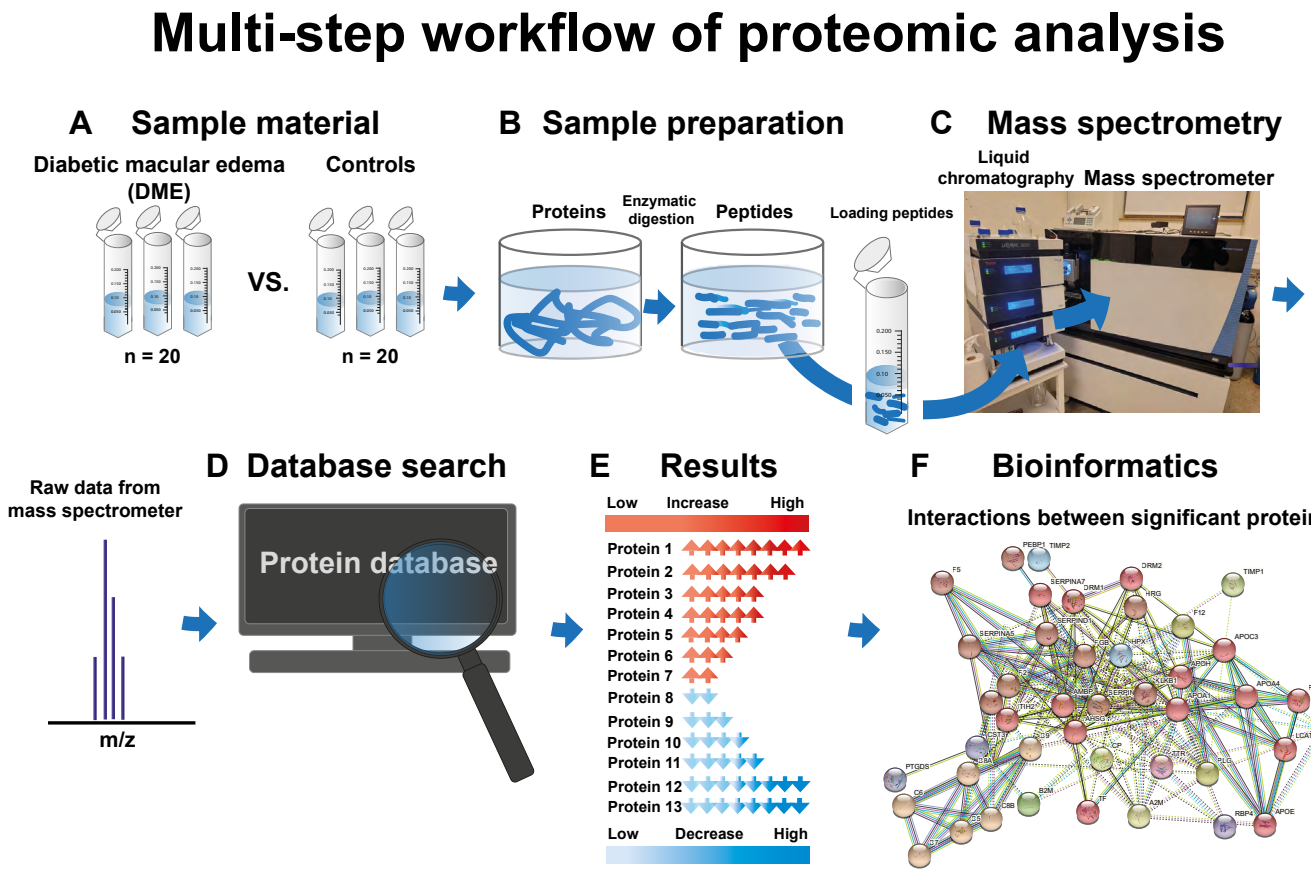


Figure 1. The successful analysis of the proteome requires a multi-step workflow. Proteomic analysis is centered around the mass spectrometer. (A) A proteome study requires a suitable sample material that allows for comparison between the disease under study and a relevant set of control samples. (B) Careful sample preparation is required. An important step is the digestion of proteins into peptides, which are used for protein quantification and identification on the mass spectrometer. (C) The peptides are loaded onto a liquid chromatography system, which separates them to allow for the identification of a greater number of proteins. After separation, peptides are passed on to the mass spectrometer. (D) Raw data are searched against existing databases to identify proteins present in the samples. (E) The output is a long list of proteins, which are either increased or decreased in the disease under study. (F) Significantly changed proteins can be grouped according to their functions and interactions to provide insights into biological processes that change in the disease under study.

spectrometric analysis. A crucial step in the sample preparation is enzymatic digestion of proteins into peptides that are unique for a given protein. The peptides are first separated on a liquid chromatography system to achieve better coverage of the proteome. Next, they are loaded onto the mass spectrometer, which measures the mass to charge ratio (m/z), and a number of peptides are specifically selected for protein quantification. To identify the proteins in the samples, the data from the mass spectrometer are searched against large protein databases. Proteins identified through this analysis that are significantly changed in DME can be correlated with clinical parameters, such as best corrected visual acuity and severity of macular edema (**Figure 2**). Key proteins identified with proteomics can be confirmed with other quantitative techniques and further tested in disease models (**Figure 2**).

At the current stage, proteomic analyses of aqueous samples from patients with DME and age-matched controls are performed in the proteomics laboratory headed by Professor Henrik Vorum. Our preliminary data show an in-depth coverage of the intraocular protein profile of DME, identifying more than 1,000 proteins with different biological functions. The identified proteins suggest that numerous biological processes contribute to the formation of DME including acute-phase response, complement activation, blood coagulation, and cholesterol metabolism. The intraocular protein profile also reflects the compromised function of glycolysis and gluconeogenesis in patients with DME.

In our next project, we are studying the aqueous proteome during anti-VEGF treatment of DME. In the first sets of samples, we predominantly observed changes in the intraocular protein profile after three or more anti-VEGF injections. Thus, the proteome analysis may indicate that at least three injections are needed to reverse the molecular mechanisms which lead to the formation of DME.

In summary, proteome analysis of DME is bringing new insights into the pathological processes underlying DME. We hope that our results can be used to improve the management of DME and contribute to the development of personalized treatment approaches.

Key points:

- Proteomic analysis can bring novel insights into disease mechanisms leading to diabetic macular edema (DME).
- With proteomics, we identified more than 1000 proteins in aqueous humor samples from patients with DME.
- Our results suggest that acute-phase response, complement activation, coagulative changes, and cholesterol metabolism contribute to the formation of DME.
- Preliminary data indicate that at least three anti-VEGF injections are needed to reverse protein changes that lead to DME.

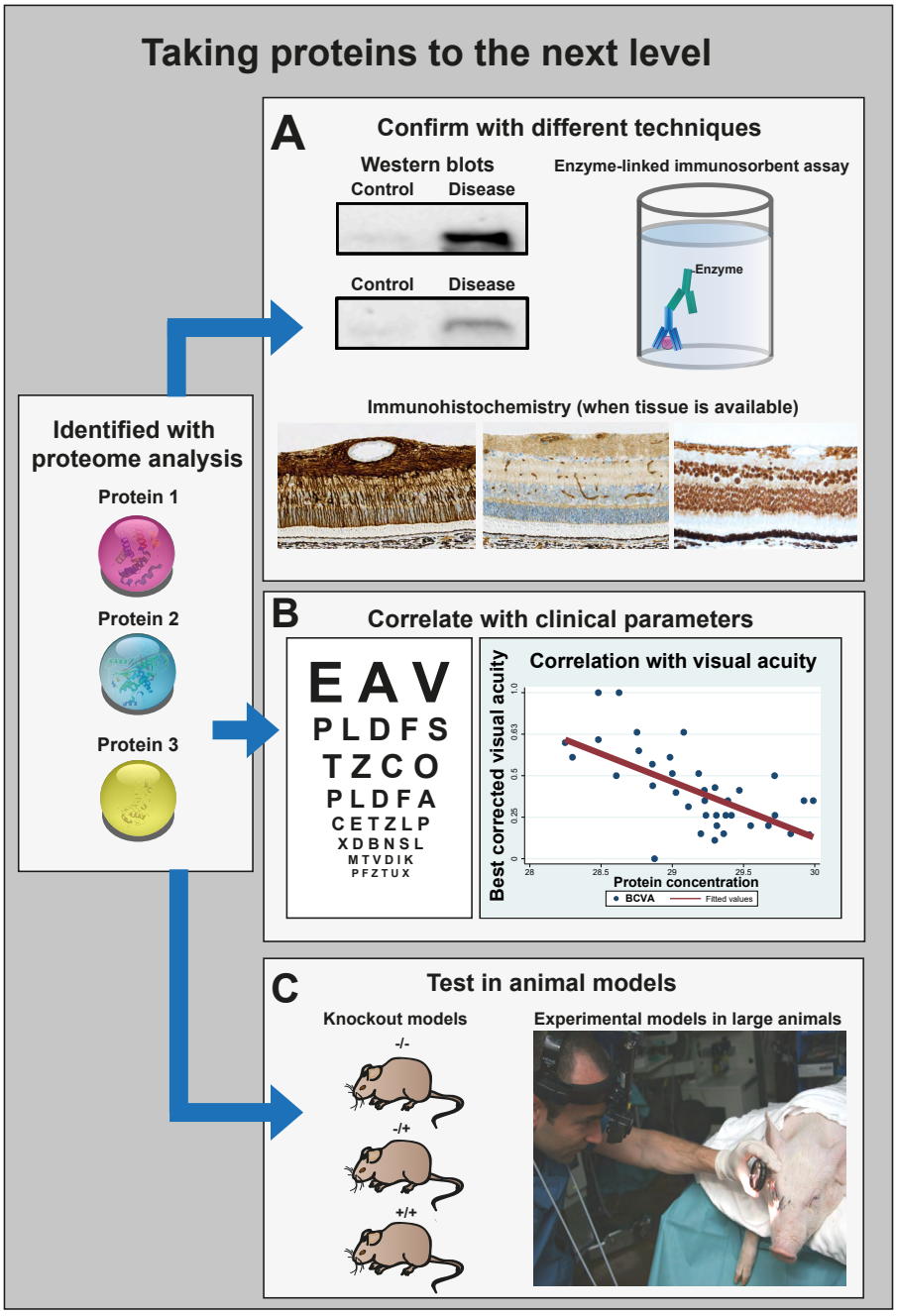


Figure 2. Proteins discovered in proteomic studies can be confirmed and further elucidated in several ways. (A) Key proteins that are significantly changed can be confirmed with other quantitative techniques, such as Western blot and enzyme-linked immunosorbent assay (ELISA). If tissue samples are available from biobanks or animal models, immunohistochemistry can be used to detect the anatomical location of the proteins. In the images provided, high concentrations of the proteins are indicated by intense brown color. (B) Significantly changed proteins can be correlated with clinical parameters such as visual acuity or severity of macular edema. (C) Identified proteins can be further explored using knockout animal models. Some disease models of ocular conditions are available in large animals.

References

1. Cehofski LJ, Kojima K, Terao N, et al. Aqueous Fibronectin Correlates With Severity of Macular Edema and Visual Acuity in Patients With Branch Retinal Vein Occlusion: A Proteome Study. *Invest Ophthalmol Vis Sci* 2020;61:6.